

Supplementary Information for:**CD25 Expression Status Improves Prognostic Risk Classification in AML****Independent of Established Biomarkers: ECOG Phase III Trial, E1900.**

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Running Head: Prognostication in Adult AML Based on CD25 Expression and Genetics

Supplemental Tables

Supplemental Table 1: Baseline Demographic and Clinical Characteristics by CD25 Expression in the Entire E1900 Cohort

Supplemental Table 2: Gene Signature Table with Differentially Expressed Genes in CD25 Positive AML Blasts. List of differentially expressed genes between CD25 positive and CD25 negative cases at log2 fold change >1 and FDR < 0.01.

Supplemental Figures

Supplemental Figure 1: Kaplan-Meier Estimates of Overall Survival, by CD25 Expression in E1900 Patients.

Supplemental Figure 2: Kaplan-Meier Estimates of Overall Survival for Intermediate-risk Patients by Cytogenetic Classification.

Supplemental Figure 3: Mutation Map for CD25 Positive Patients.

Supplemental Figure 4: Kaplan-Meier Estimates of Overall Survival by Mutation Status and Daunorubicin Dose, by CD25 Expression.

Supplemental Tables

Supplemental Table 1: Baseline Demographic and Clinical Characteristics by CD25 Expression in the Entire E1900 Cohort

	CD25 Negative (n=570)	CD25 Positive (n=87)	p-value
	Median (Range)	Median (Range)	
Age	48 (17-60)	48 (18-60)	0.66
WBC (1000/mm ³)	10.1 (0.6-366.0)	23.6 (0.8-181.0)	<0.001
Hemoglobin (g/dl)	9.2 (4.6-30.0)	9.3 (5.1-12.7)	0.49
Peripheral Blasts (%)	30 (0-90)	48.5 (0-94)	0.001
Platelets (1000/mm ³)	53.0 (0.7-995.0)	52.0 (3.9-261.0)	0.78
Marrow Blast (%)	64.5(3.0-100.0)	64.0(9.0-99.0)	0.88
	# Patients (%)	# Patients (%)	
Female Sex	271 (47.5%)	51 (58.6%)	0.07
ECOG PS 0	266 (67.7%)	38 (43.7%)	
1	267 (46.8%)	40 (46.0%)	
2+	33 (5.8%)	8 (9.1%)	0.16
Cytogenetics Favorable	87 (15.3%)	2 (2.3%)	<0.0001
Cytogenetics Intermediate	207 (36.3%)	80 (92.0%)	
Cytogenetics Unfavorable	117 (20.5%)	5 (5.7%)	
Secondary AML	15 (2.6%)	7 (8.0%)	0.02

WBC, white blood cell count; PS, performance status; AML, acute myeloid leukemia;

Supplemental Table 2: Gene Signature Table with Differentially Expressed Genes in CD25 Positive AML Blasts. List of differentially expressed genes between CD25 positive and CD25 negative cases at log2 fold change >1 and FDR < 0.01.

SEQ_ID	ACCESSION	CHROM	STRAND	TXSTART	TXEND	SYMBOL	DESCRIPTION	
HSAP0406S00000157	NM_198543	chr1	-	3003792	3007490	MGC35434	LOC148872 protein	Homo sapiens LOC148872 protein (MGC35434), mRNA.
HSAP0406S00001779	NM_002959	chr1	-	109564233	109652605	SORT1	sortilin 1 preproprotein	Homo sapiens sortilin 1 (SORT1), mRNA.
HSAP0406S00002177	NM_019032	chr1	+	147334957	147346481	TSRC1	thrombospondin repeat containing 1	Homo sapiens thrombospondin repeat
HSAP0406S00002179	BC027478	chr1	+	147341015	147346481	TSRC1	TSRC1 protein	Homo sapiens thrombospondin repeat containing 1, mRNA (cDNA clone IMAGE:5001173),
HSAP0406S00004472	NM_003494	chr2	+	71592414	71825547	DYSF	dysferlin	Homo sapiens dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive) (DYSF),
HSAP0406S00004473	AK074104	chr2	+	71673833	71825547	FLJ00175	FLJ00175 protein	Homo sapiens mRNA for FLJ00175 protein.
HSAP0406S00005562	NM_004657	chr2	-	192525246	192537296	SDPR	serum deprivation response protein	Homo sapiens serum deprivation response (phosphatidylserine binding
HSAP0406S00005612	NM_015535	chr2	+	200996301	201168758	DNAPTP6	DNA polymerase-transactivated protein 6	Homo sapiens DNA polymerase-transactivated protein 6 (DNAPTP6), mRNA.
HSAP0406S00005613	AF193059	chr2	+	200996936	201168758	SP1224	unknown	Homo sapiens SP1224 mRNA, complete cds.
HSAP0406S00005785	NM_000597	chr2	+	217323638	217354662	IGFBP2	insulin-like growth factor binding protein 2 (36kD)	Homo sapiens insulin-like growth factor binding protein 2 (36kD) (IGFBP2), mRNA.
HSAP0406S00006956	D87433	chr3	+	52504410	52533549	KIAA0246	KIAA0246 protein	Homo sapiens mRNA for KIAA0246 protein, partial cds.
HSAP0406S00006957	AB052956	chr3	+	52504414	52533549	FELE-1	FELE-1	Homo sapiens mRNA for FELE-1, complete cds.

HSAP0406S00007128	BC031289	chr3	-	71814670	71842326	EIF4E3	EIF4E3 protein	Homo sapiens eukaryotic translation initiation factor 4E member 3, mRNA (cDNA clone IMAGE:5299499), partial cds.
HSAP0406S00007130	NM_021935	chr3	-	71903496	71916902	PROK2	prokineticin 2	Homo sapiens prokineticin 2 (PROK2), mRNA.
HSAP0406S00007258	NM_001627	chr3	+	106568879	106776709	ALCAM	activated leukocyte cell adhesion molecule	Homo sapiens activated leukocyte cell adhesion molecule (ALCAM), mRNA.
HSAP0406S00007291	NM_018189	chr3	-	110527890	110539083	DPPA4	developmental pluripotency associated 4	Homo sapiens developmental pluripotency associated 4 (DPPA4), mRNA.
HSAP0406S00007585	NM_007117	chr3	+	131176260	131179478	TRH	thyrotropin-releasing hormone	Homo sapiens thyrotropin-releasing hormone (TRH),
HSAP0406S00007761	BC012613	chr3	+	150065781	150097566	CPA3	mast cell carboxypeptidase A3, precursor	Homo sapiens carboxypeptidase A3 (mast cell), mRNA (cDNA clone MGC:13637 IMAGE:4104766),
HSAP0406S00007812	BC031063	chr3	-	152637011	152641266	IGSF10	IGSF10 protein	Homo sapiens immunoglobulin superfamily, member 10, mRNA (cDNA clone IMAGE:5271204), complete cds.
HSAP0406S00007815	NM_178822	chr3	-	152637174	152659195	IGSF10	immunoglobulin superfamily, member 10	Homo sapiens immunoglobulin superfamily, member 10 (IGSF10), mRNA.
HSAP0406S00008550	BC009945	chr4	-	25425396	25497831	KIAA0746	KIAA0746 protein	Homo sapiens KIAA0746 protein, mRNA (cDNA clone IMAGE:4123572), partial cds.
HSAP0406S00008551	NM_015187	chr4	-	25425318	25540713	KIAA0746	KIAA0746 protein	Homo sapiens KIAA0746 protein (KIAA0746), mRNA.
HSAP0406S00008552	AB018289	chr4	-	25425329	25525610	KIAA0746	KIAA0746 protein	Homo sapiens mRNA for KIAA0746 protein, partial cds.

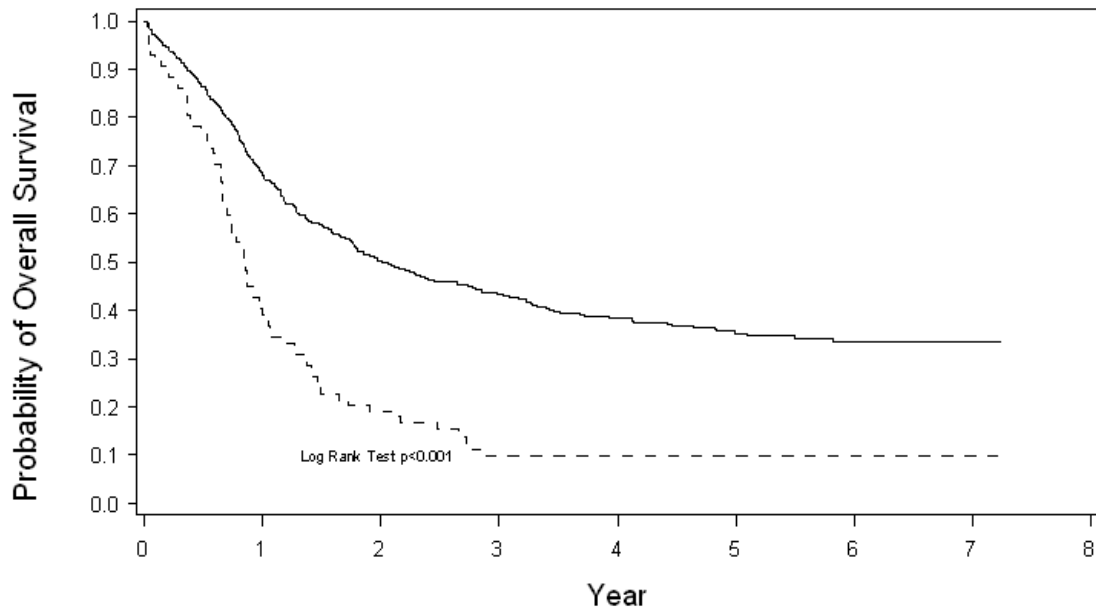
HSAP0406S00008732	BC022514	chr4	-	57516954	57528825	SPINK2	serine protease inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)	Homo sapiens serine protease inhibitor, Kazal type 2 (acrosin-trypsin inhibitor), mRNA (cDNA clone MGC:26562 IMAGE:4812189), complete
HSAP0406S00008798	NM_144646	chr4	-	71886718	71897299	IGJ	immunoglobulin J chain	Homo sapiens immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides (IGJ), mRNA.
HSAP0406S00008840	NM_002090	chr4	-	75267348	75269525	CXCL3	chemokine (C-X-C motif) ligand 3	Homo sapiens chemokine (C-X-C motif) ligand 3 (CXCL3),
HSAP0406S00009434	Y15723	chr4	+	156945482	157009379	sGC	soluble guanylyl cyclase	Homo sapiens mRNA for soluble guanylyl cyclase.
HSAP0406S00009912	NM_005410	chr5	-	42835746	42847717	SEPP1	selenoprotein P precursor	Homo sapiens selenoprotein P, plasma, 1 (SEPP1), mRNA.
HSAP0406S00011216	BC027302	chr5	-	179961128	179962286	LOC285682	LOC285682 protein	Homo sapiens hypothetical protein LOC285682, mRNA (cDNA clone IMAGE:4212883),
HSAP0406S00011217	S66407	chr5	-	179962797	179968590	FLT4	receptor tyrosine kinase isoform FLT4 long	FLT4=receptor tyrosine kinase isoform FLT4 long {3' region, alternatively spliced} [human, mRNA Partial, 216 nt].
HSAP0406S00012409	BX641095	chr6	+	74462642	74590174	DKFZp686N23150	hypothetical protein	Homo sapiens mRNA
HSAP0406S00012807	NM_004666	chr6	-	133044422	133076881	VNN1	vanin 1 precursor	Homo sapiens vanin 1 (VNN1), mRNA.
HSAP0406S00013480	NM_019102	chr7	-	26954235	26956527	HOXA5	homeobox protein A5	Homo sapiens homeo box A5 (HOXA5), mRNA.
HSAP0406S00013483	NM_152739	chr7	-	26975297	26978389	HOXA9	homeobox protein A9 isoform a	Homo sapiens homeo box A9 (HOXA9), transcript variant 1, mRNA.
HSAP0406S00013484	NM_018951	chr7	-	26983450	26987163	HOXA10	homeobox protein A10 isoform a	Homo sapiens homeo box A10 (HOXA10), transcript variant 1, mRNA.

HSAP0406S00013485	NM_153715	chr7	-	26983450	26993083	HOXA10	homeobox protein A10 isoform b	Homo sapiens homeo box A10 (HOXA10), transcript variant 2, mRNA.
HSAP0406S00013498	NM_019029	chr7	-	28808492	28959353	CPVL	serine carboxypeptidase vitellogenic-like	Homo sapiens carboxypeptidase, vitellogenic-like (CPVL), transcript variant 2,
HSAP0406S00015750	NM_018407	chr8	+	98856984	98933998	LAPTM4B	lysosomal-associated transmembrane protein 4 beta	Homo sapiens lysosomal associated protein transmembrane 4 beta
HSAP0406S00015751	AJ276485	chr8	+	98857341	98933998	LC27	integral membrane transporter protein	Homo sapiens mRNA for putative integral membrane transporter protein (LC27
HSAP0406S00015866	NM_006209	chr8	-	120638506	120720251	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	Homo sapiens ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) (ENPP2),
HSAP0406S00016747	NM_000689	chr9	-	72745141	72797523	ALDH1A1	aldehyde dehydrogenase 1A1	Homo sapiens aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), mRNA.
HSAP0406S00017066	NM_080546	chr9	+	105086662	105233235	CDW92	CDW92 antigen	Homo sapiens CDW92 antigen (CDW92), mRNA.
HSAP0406S00017067	BC018213	chr9	+	105208200	105232183	CDW92	CDW92 protein	Homo sapiens CDW92 antigen, mRNA (cDNA clone IMAGE:3882479), partial cds.
HSAP0406S00017533	NM_0010022	chr9	+	131001465	131028093	C9orf58	chromosome 9 open reading frame 58 isoform 2	Homo sapiens chromosome 9 open reading frame 58 (C9orf58), transcript variant 2,
HSAP0406S00017894	NM_000417	chr10	-	6093511	6144278	IL2RA	interleukin 2 receptor, alpha chain precursor	Homo sapiens interleukin 2 receptor, alpha (IL2RA), mRNA.
HSAP0406S00017997	NM_002438	chr10	+	17891367	17993183	MRC1	mannose receptor C type 1 precursor	Homo sapiens mannose receptor, C type 1 (MRC1),
HSAP0406S00017998	NM_002438	chr10	+	18138357	18240096	MRC1	mannose receptor C type 1 precursor	Homo sapiens mannose receptor, C type 1 (MRC1),

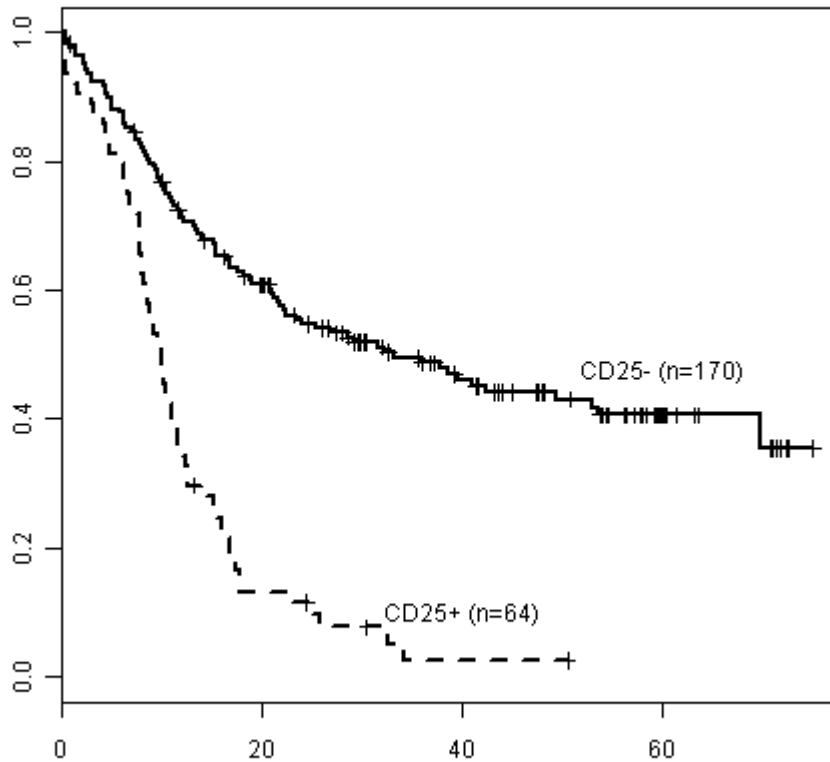
HSAP0406S00018221	BC004376	chr10	-	46577996	46594046	ANXA8	annexin A8	Homo sapiens annexin A8, mRNA (cDNA clone MGC:10570 IMAGE:3639064), complete
HSAP0406S00018223	BC004376	chr10	+	47216995	47233039	ANXA8	annexin A8	Homo sapiens annexin A8, mRNA (cDNA clone MGC:10570 IMAGE:3639064), complete
HSAP0406S00018227	BC004376	chr10	+	47875312	47891367	ANXA8	annexin A8	Homo sapiens annexin A8, mRNA (cDNA clone MGC:10570 IMAGE:3639064), complete
HSAP0406S00018850	NM_145285	chr10	+	101282699	101286268	NKX2-3	NK2 transcription factor related, locus 3	Homo sapiens NK2 transcription factor related, locus 3 (Drosophila) (NKX2-3),
HSAP0406S00019211	NM_001380	chr10	+	128658954	129140769	DOCK1	dedicator of cytokinesis 1	Homo sapiens dedicator of cytokinesis 1 (DOCK1), mRNA.
HSAP0406S00021250	NM_153206	chr11	-	117569887	117589284	AMICA	adhesion molecule AMICA	Homo sapiens adhesion molecule AMICA (AMICA),
HSAP0406S00021925	U21551	chr12	-	24862208	24946087	ECA39	N/A	Human ECA39 mRNA,
HSAP0406S00022829	NM_003877	chr12	+	92466065	92472446	SOCS2	suppressor of cytokine signaling-2	Homo sapiens suppressor of cytokine signaling 2 (SOCS2),
HSAP0406S00023979	NM_024089	chr13	-	102234632	102249358	KDEL1	KDEL (Lys-Asp-Glu-Leu) containing 1	Homo sapiens KDEL (Lys-Asp-Glu-Leu) containing 1 (KDEL1),
HSAP0406S00024319	NM_001911	chr14	-	24112563	24115306	CTSG	cathepsin G preproprotein	Homo sapiens cathepsin G (CTSG), mRNA.
HSAP0406S00024524	NM_000161	chr14	-	54378484	54439279	GCH1	GTP cyclohydrolase 1 (dopa-responsive dystonia)	Homo sapiens GTP cyclohydrolase 1 (dopa-responsive dystonia) (GCH1),
HSAP0406S00025293	NM_014792	chr14	+	105461656	105469554	KIAA0125	KIAA0125 gene product	Homo sapiens KIAA0125 (KIAA0125), mRNA.
HSAP0406S00025500	NM_003246	chr15	+	37660571	37676959	THBS1	thrombospondin 1 precursor	Homo sapiens thrombospondin 1 (THBS1),
HSAP0406S00025666	NM_002373	chr15	+	41597132	41611109	MAP1A	microtubule-associated protein 1A	Homo sapiens microtubule-associated protein 1A (MAP1A), mRNA.

HSAP0406S00026591	BC003512	chr16	+	750767	758866	MSLN	megakaryocyte potentiating factor, precursor	Homo sapiens mesothelin, transcript variant 1, mRNA (cDNA clone MGC:10686 IMAGE:3611296), complete
HSAP0406S00026616	NM_012217	chr16	+	1246273	1248495	TPSD1	tryptase delta 1	Homo sapiens tryptase delta 1 (TPSD1), mRNA.
HSAP0406S00027488	NM_004530	chr16	+	54070603	54097652	MMP2	matrix metalloproteinase 2 preproprotein	Homo sapiens matrix metalloproteinase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase) (MMP2),
HSAP0406S00027569	NM_201524	chr16	+	56220041	56256445	GPR56	G protein-coupled receptor 56 isoform b	Homo sapiens G protein- coupled receptor 56 (GPR56), transcript variant 2, mRNA.
HSAP0406S00027570	NM_170776	chr16	+	56259671	56280789	GPR97	G protein-coupled receptor 97	Homo sapiens G protein- coupled receptor 97 (GPR97),
HSAP0406S00028298	NM_182538	chr17	+	4283967	4338247	MGC29671	hypothetical protein MGC29671	Homo sapiens hypothetical protein MGC29671
HSAP0406S00028299	BC023646	chr17	+	4284015	4338247	MGC29671	MGC29671 protein	Homo sapiens hypothetical protein MGC29671, mRNA (cDNA clone MGC:29671 IMAGE:5087991), complete
HSAP0406S00029491	U59298	chr17	-	43982349	43986269	HOXB3	hox homeobox transcription factor HOXB3	Human hox homeobox transcription factor (HOXB3) mRNA, complete cds.
HSAP0406S00029492	NM_024015	chr17	-	44007874	44010742	HOXB4	homeo box B4	Homo sapiens homeo box B4 (HOXB4), mRNA.
HSAP0406S00029493	M92299	chr17	-	44023618	44026322	HOX2A	homeobox protein	Human homeobox 2.1 protein (HOX2A) mRNA, complete cds.
HSAP0406S00029604	NM_005486	chr17	+	50333202	50394309	TOM1L1	target of myb1-like 1	Homo sapiens target of myb1- like 1 (chicken) (TOM1L1),
HSAP0406S00029646	NM_006151	chr17	+	53670846	53700878	LPO	lactoperoxidase	Homo sapiens lactoperoxidase (LPO), mRNA.
HSAP0406S00029647	BX538344	chr17	+	53681457	53700794	DKFZp686G24255	hypothetical protein	Homo sapiens mRNA

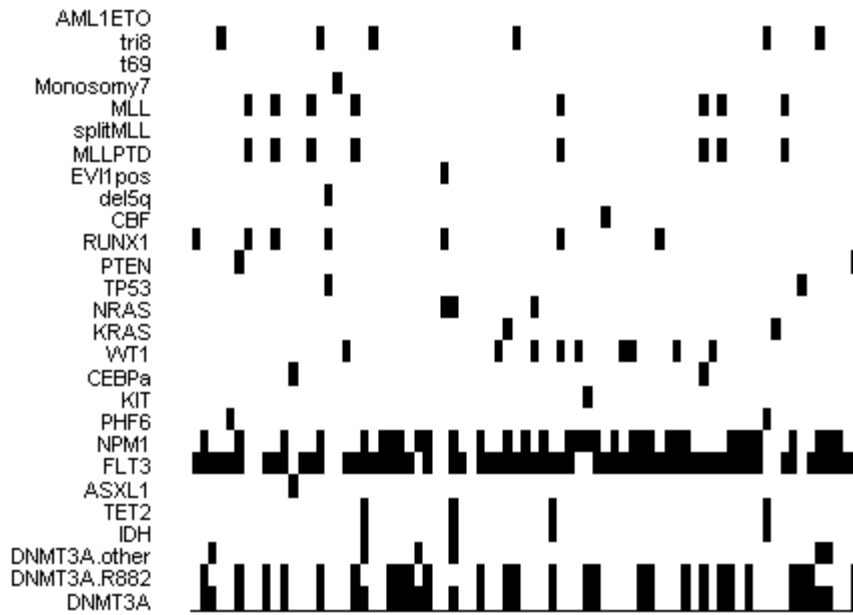
HSAP0406S00030121	NM_005567	chr17	-	74478931	74487656	LGALS3BP	galectin 3 binding protein	Homo sapiens lectin, galactoside-binding, soluble, 3 binding protein (LGALS3BP),
HSAP0406S00030392	NM_006868	chr18	+	9698302	9852547	RAB31	RAB31, member RAS oncogene family	Homo sapiens RAB31, member RAS oncogene family (RAB31),
HSAP0406S00030688	NM_003199	chr18	-	51045966	51406441	TCF4	transcription factor 4 isoform b	Homo sapiens transcription factor 4 (TCF4), mRNA.
HSAP0406S00031124	NM_020209	chr19	+	4230658	4241720	SHD	src homology 2 domain-containing transforming protein D	Homo sapiens src homology 2 domain-containing transforming protein D (SHD),
HSAP0406S00031248	NM_001974	chr19	+	6838581	6891463	EMR1	egf-like module containing, mucin-like, hormone receptor-like	Homo sapiens egf-like module containing, mucin-like, hormone receptor-like 1
HSAP0406S00035974	NM_0010053	chrX	+	51469733	51478484	MAGED1	melanoma antigen, family D, 1 isoform a	Homo sapiens melanoma antigen, family D, 1 (MAGED1), transcript variant 1, mRNA.
HSAP0406S00036380	NM_033381	chrX	+	107489298	107746920	COL4A5	alpha 5 type IV collagen isoform 3, precursor	Homo sapiens collagen, type IV, alpha 5 (Alport syndrome) (COL4A5), transcript variant 3,
HSAP0406S00036381	M58526	chrX	+	107613275	107745753	COL4A5	alpha-5 type IV collagen	Human alpha-5 collagen type IV (COL4A5) mRNA, 3' end.
HSAP0406S00036590	NM_001449	chrX	+	134955199	135019028	FHL1	four and a half LIM domains 1	Homo sapiens four and a half LIM domains 1 (FHL1), mRNA.
HSAP0406S00036702	NM_007150	chrX	+	151757232	151812583	ZNF185	zinc finger protein 185 (LIM domain)	Homo sapiens zinc finger protein 185 (LIM domain)
HSAP0406S00036703	BX640940	chrX	+	151759471	151812583	DKFZp686B22130	hypothetical protein	Homo sapiens mRNA
HSAP0406S00036704	BX537525	chrX	+	151770641	151812589	DKFZp686O1389	hypothetical protein	Homo sapiens mRNA
HSAP0406S00036818	BC005367	chrX	-	154070161	154127570	CLIC2	CLIC2 protein	Homo sapiens chloride intracellular channel 2, mRNA (cDNA clone MGC:12482 IMAGE:3930673), complete
HSAP0406S00036819	NM_001289	chrX	-	154070159	154127661	CLIC2	chloride intracellular channel 2	Homo sapiens chloride intracellular channel 2 (CLIC2),

Supplemental Figures

Supplemental Figure 1: Kaplan-Meier Estimates of Overall Survival, by CD25 Expression in E1900 Patients. Data from the intention-to-treat analysis are shown for the entire E1900 cohort (n=657). CD25 negative patients are depicted in the solid curve (N=570), while CD25 positive patients are depicted as a dashed curve (N=87).

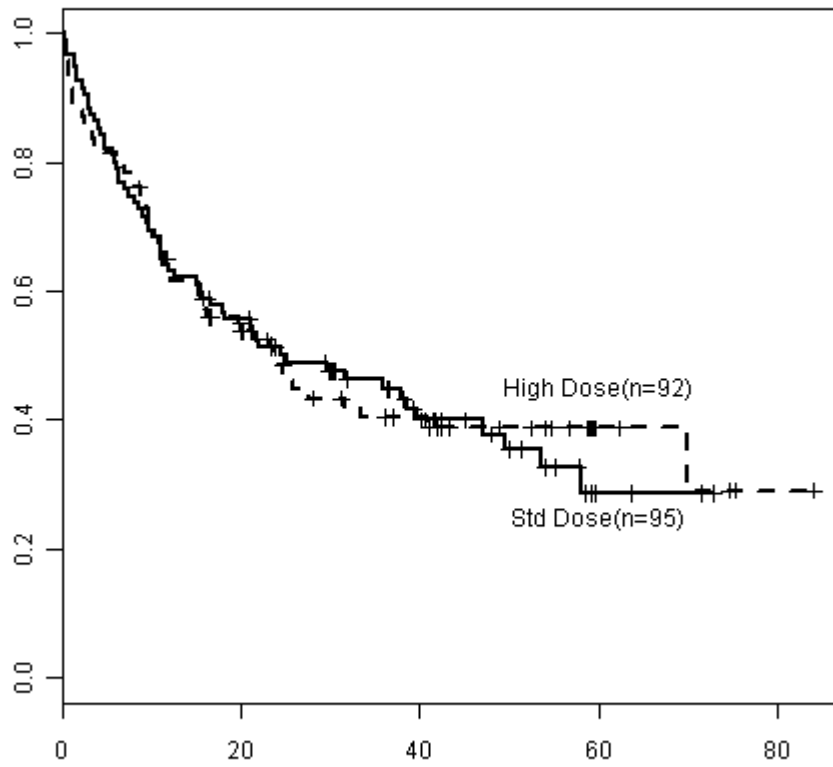


Supplemental Figure 2: Kaplan-Meier Estimates of Overall Survival for Intermediate-risk Patients by Cytogenetic Classification. Data are shown for cytogenetically intermediate-risk patients either lacking CD25 (solid line, N=170) or expressing CD25 (dashed line, N=64).

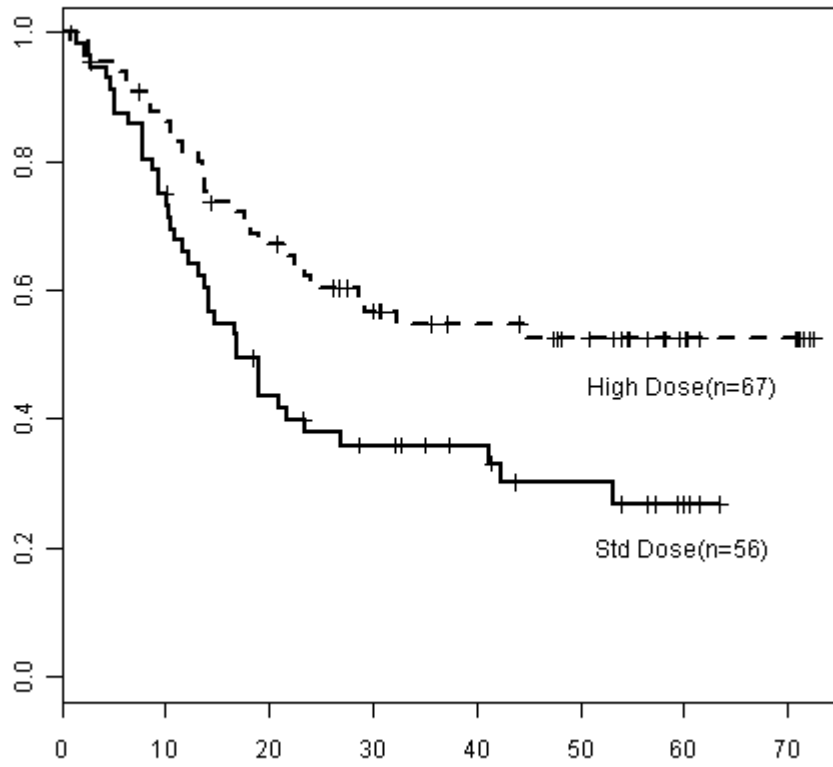


Supplemental Figure 3: Mutation Map for CD25 Positive Patients. Data are depicted for all CD25 positive patients (N=75). Each column is a patient and each row is one of the 27 mutations analyzed. Black denotes mutation and white denotes wild type.

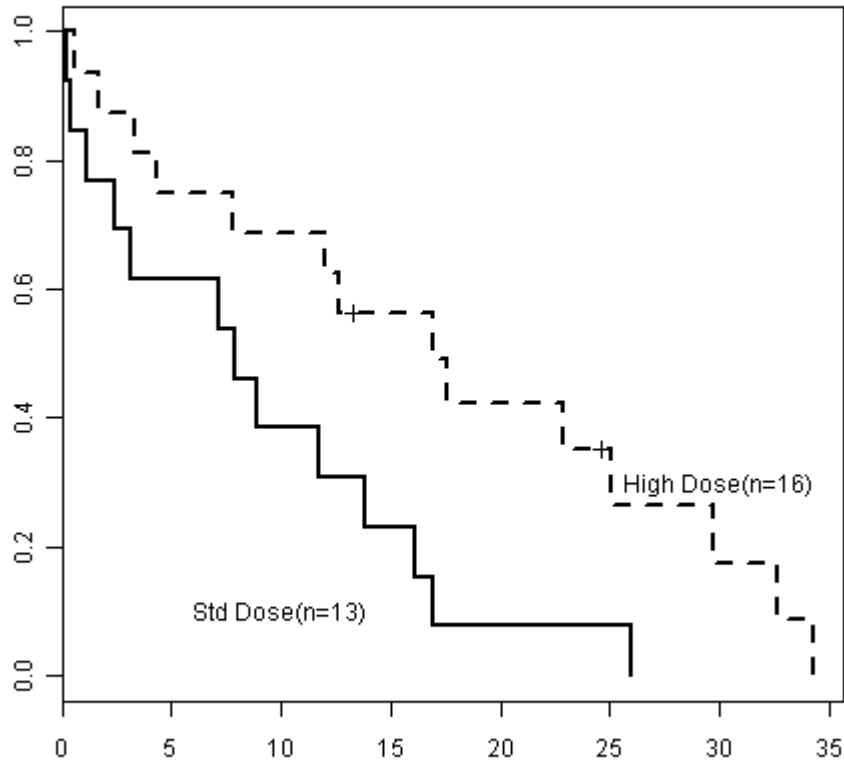
Supplemental Figure 4A:

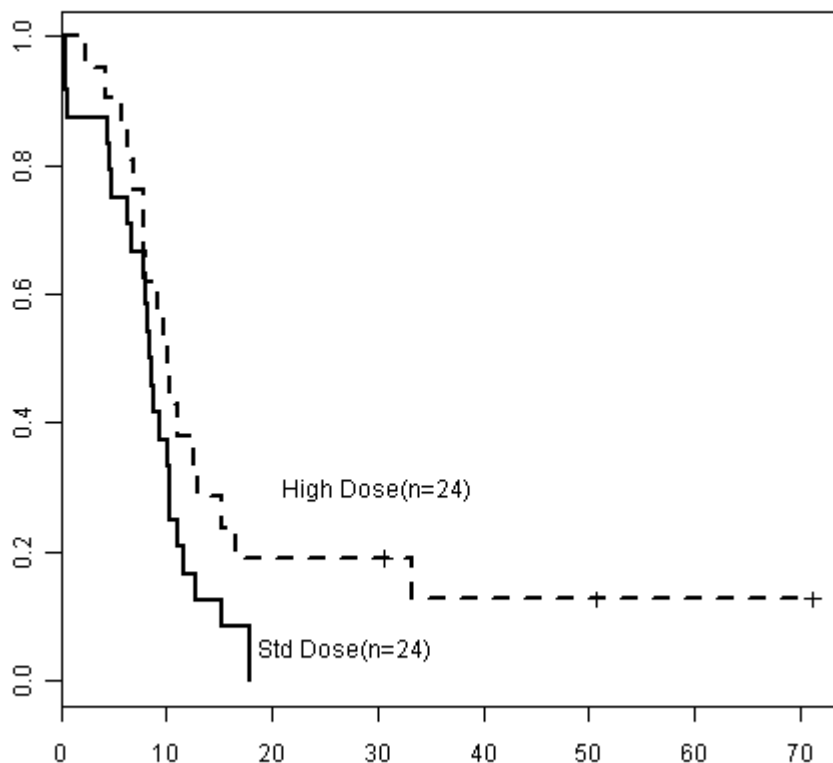


Supplemental Figure 4B:



Supplemental Figure 4C:



Supplemental Figure 4D:

Supplemental Figure 4: Kaplan-Meier Estimates of Overall Survival by Mutation Status and Daunorubicin Dose. In each panel, the solid line represents high-dose daunorubicin, while the dashed line represents standard-dose daunorubicin. Data are shown for CD25 negative patients (Panel A and B), wild-type for DNMT3A and NPM1, and without chromosome translocations involving the MLL gene (SDD: N=95, HDD: N=92) ($p=0.931$) (**Panel A**) or with at least one mutation in DNMT3A or NPM1

or with MLL translocation (SDD: N=56, HDD: N=67) ($p=0.006$) (**Panel B**). In Panel C and D, data are depicted for CD25 positive patients who were either wild-type for DNMT3A and NPM1 (SDD: N=13, HDD: N=16) ($p=0.002$) (**Panel C**) or with at least one mutation in DNMT3A or NPM1 (SDD: N=24, HDD: N=21) ($p=0.067$) (**Panel D**). None of the CD25 positive patients had chromosomal translocations involving the MLL gene.